

Tris-HCl buffer solution (pH = 8.5) containing 100 mM NaCl, 3 mM calcium chloride, 0.1 % bovine serum albumin (supplied from the firm Sigma) and 0.225 NIHU of human thrombin (supplied from the firm Sigma) and the mixture is stood still for 15 minutes at 37 °C, where to 7.5  $\mu$  l of bovine protein C of about 300  $\mu$  g/ml (supplied from the firm Life Technologies) are added and the resulting mixture is again stood still for 30 minutes at 37 °C in order to activate the protein C. Then, about 7.5  $\mu$  l of an aqueous solution containing about 100  $\mu$  l/ml of a heparin (supplied from Wako Pure Chemical Ind., Ltd.) and about 6  $\mu$  l/ml of Antithrombin III (of the firm Life Technologies) are added to the mixture to terminate the reaction. To this mixture are then added 500  $\mu$  l of a solution containing 100  $\mu$  g/ml of a synthetic substrate (Boc-Leu-Ser-Thr-Arg-MCA) (SEQ ID NO: 6) and the resulting mixture is stood still for 20 minutes at 37°C. The substrate-scissoring reaction is then terminated by adding 50  $\mu$  l of acetic acid. The reaction mixture is examined by observing the fluorescence strength at an excitation wave length of 380 nm and at an emission wave length of 440 nm using a fluorescence spectrophotometer to determine the amount of the existing activated protein C, whereupon the thrombomodulin activity is calculated by comparison with a reference preparation of standard thrombomodulin activity.--

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